PCT Application (Priority EP 02 017 953.7) Applicant: Max-Planck-Gesellschaft zur ... PCT August 4, 2003 Our Ref: GI-012

CLAIMS

- 1. Transcriptionally inactivated (caged) tetracycline or tetracycline derivative, wherein the inactivation is caused by reaction of the tetracycline (derivative) with a photosensitive protection compound, said caged tetracycline or tetracycline derivative being capable to be activated again by photolysis.
- 2. Transcriptionally inactivated (caged) tetracycline or tetracycline derivative according to claim 1, wherein said derivative is doxycycline, anhydro-tetracycline, or minocycline.
- 3. Transcriptionally inactivated (caged) tetracycline or tetracycline derivative according to claim 1 or 2, wherein said tetracycline or tetracycline derivative contains a functional group such as an amino, an amide, a carbonyl, a sulfhydryl, or preferably a hydroxy function.
- 4. Transcriptionally inactivated (caged) tetracycline or tetracycline derivative according to any of the preceding claims, wherein said photosensitive protection compound is a compound comprising an α-carboxy-2-nitrobenzyl (CNB), a coumarinyl, a desoxybenzoinyl, or a hydroxyphenacyl residue.
- 5. Transcriptionally inactivated (caged) tetracycline or tetracycline derivative according to any of the preceding claims, wherein said photosensitive protection compound is 1-(1-diazoethyl)-4,5-dimethoxy-2-nitrobenzene or 4-(1-diazoethyl)-7-methoxycoumarin.
- 6. Method to prepare the transcriptionally inactivated (caged) tetracycline or tetracycline derivative as defined in any of the preceding claims, the method comprising the step of reacting a compound comprising a reactive group capable to react with the functional group of the tetracycline (derivative) and a group capable to absorb electromagnetic radiation in the UV range with tetracycline or a derivative thereof.

- 7. The method according to claim 6, wherein the functional group of the tetracycline (derivative) is an amino, an amide, a carbonyl, a sulfhydryl, or a hydroxy group, and wherein the reactive group of the compound is an alkyl hydrazone/diazoalkyl, an oxiranyl, or a 1,2-dihydroxyethyl group.
- 8. The method according to claim 6 or 7, wherein either of doxycycline, anhydro-tetracycline, or minocycline is reacted with 1-(1-diazoethyl)-4,5-dimethoxy-2-nitrobenzene or 4-(1-diazoethyl)-7-methoxycoumarin.
- 9. The method according to any of claims 6 to 8, wherein the photosensitive protection compound is obtained by oxidation of a photosensitive protection precursor compound with manganese dioxide as an oxidizing agent.
- 10. Use of the transcriptionally inactivated (caged) tetracycline or tetracycline derivative as defined in any of claims 1 to 5 to induce expression of a gene/transgene either at a defined point of time or in a defined set of cells, or both.
- 11. Kit, comprising, in a suitable container means,
 - (i) either a transcriptionally inactivated (caged) tetracycline (derivative) or a transcriptionally active (uncaged) tetracycline (derivative), a photosensitive protection compound or its precursor and, optionally, an oxidizing agent; and
 - (ii)a gene vector suitable to confer tetracycline-dependent transgene expression (i.e., a vector expressing a fusion protein necessary to induce gene/transgene expression and a vector containing a tetracycline-dependent transgene)
- 12. In vitro- and in vivo-method for the controlled expression of a tetracycline-dependent gene/transgene in a defined set of cells (target cells), the method comprising the following steps:
 - (a)reacting tetracycline or a tetracycline derivative with a photosensitive protection com-pound to prepare a caged tetracycline or a caged tetracycline derivative, both as defined in any of claims 1 to 5;
 - (b)introducing, in any order of the steps, said caged tetracycline or tetracycline derivative, the tetracycline-dependent transgene, and the gene encoding a transcription factor that is inactive absent its binding to tet or a tet derivative into said defined set of cells (target cells),

wherein introduction of the transgene and the gene for the transcription factor in its inactive form is not required, provided the target cells express the transgene and the transcription factor gene, respectively; and

- (c) irrradiating the defined set of cells.
- 13. The method of claim 12, wherein the cells are irradiated with UV light.
- 14. The method of claim 12, wherein irradiation of the cells occurs via 2- or multi-photon microscopy.
- 15. The method of any of claims 12 to 14, the method further comprising the following step of (d) detecting the polypeptide (protein) expressed in the defined set of cells.